Deconvolution of Clinical Variance in CAR-T Cell Pharmacology and Response

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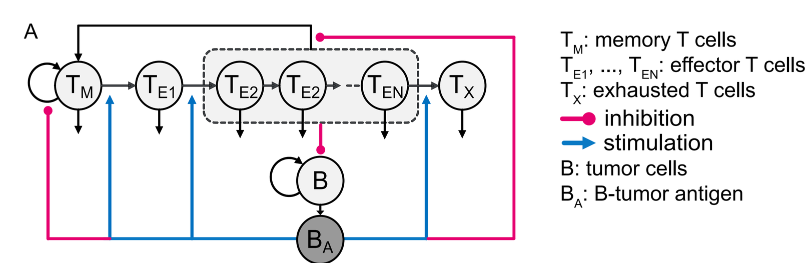
OVERVIEW

- CAR-T cell expansion and persistence varies widely between patients and is predictive of efficacy. What underlies this variance?
- We developed a mathematical model of T cell regulatory control wherein transitions between memory, effector, and exhausted T cell states are coordinately regulated by antigen engagement. We trained this model on clinical data in different hematological malignancies and identified cell-intrinsic differences in the turnover rate of memory cells and the cytotoxic potency of effectors as the primary determinants of exposure and response.
- Pre-infusion product transcriptomics confirm these results and predict patient outcomes to CD19 CAR-T therapy with better accuracy than standard immunophenotyping.
- Mathematical modelling predicts, de novo, clinical variance in exposure, covariates of response, and the biological mechanisms underlying the pharmacology of CAR-Ts across multiple indications.

An antigen toggle-switch model of T cell regulation quantitatively describes PKPD behavior of complete, partial, and non-esponding (CR, PR, and NR) patient population responses to Kymriah in CLL

Model structure, parametrization, and mechanisms differentiating CR, PR, and NR populations.



- **A** Cartoon depiction of the model structure.
- **B** Model fits agree with reported[1] CAR-T and B-cell tumor dynamics for CR, PR, and NR populations.
- **C** PCA plot based on the logarithm of the best fitting parameters colored by population.
- **D** Sorted PC-1 coefficients suggest that $TK50$ (threshold for killing) and μ_M and d_M (T memory cell turnover) are the largest sources of variation between CR and NR populations.

METHODS

- Pharmacokinetic and tumor dynamic profiles were digitized from literature, and PK data for Kymriah in B-ALL was generated using a published non-linear mixed effects model by Stein et al.[2]
- The toggle-switch model was encoded as a system of nonlinear ordinary differential equations, and parameters were estimated using particle swarm optimization. Virtual populations were generated by Monte Carlo sampling parameters with random CAR-T dose and initial tumor burden.
- Bulk RNA sequencing data[1] was TMM normalized and converted to log(counts per million) by Voom transformation. Differential gene expression was implemented with Limma and gene signature enrichment estimated with single sample GSEA. Gene signatures for cell signaling pathways were compiled from PROGENy, BioCarta, Reacome, Hallmark, and David, and T cell populations signatures from Fraietta et al.[1] and the cell atlas of human thymic development.[3] Single-cell RNA sequencing was obtained from Bai et al.[4] and Haradhvala et al.[5] and normalized with Seurat and annotated with ProjecTILs.[6]

1. Fraietta, J. A. et al. Nat Med 24, 563–571 (2018).
2. Stein, A. M. et al. Cpt Pharmacometrics Syst Pharmacol 8, 285–295 (2019).
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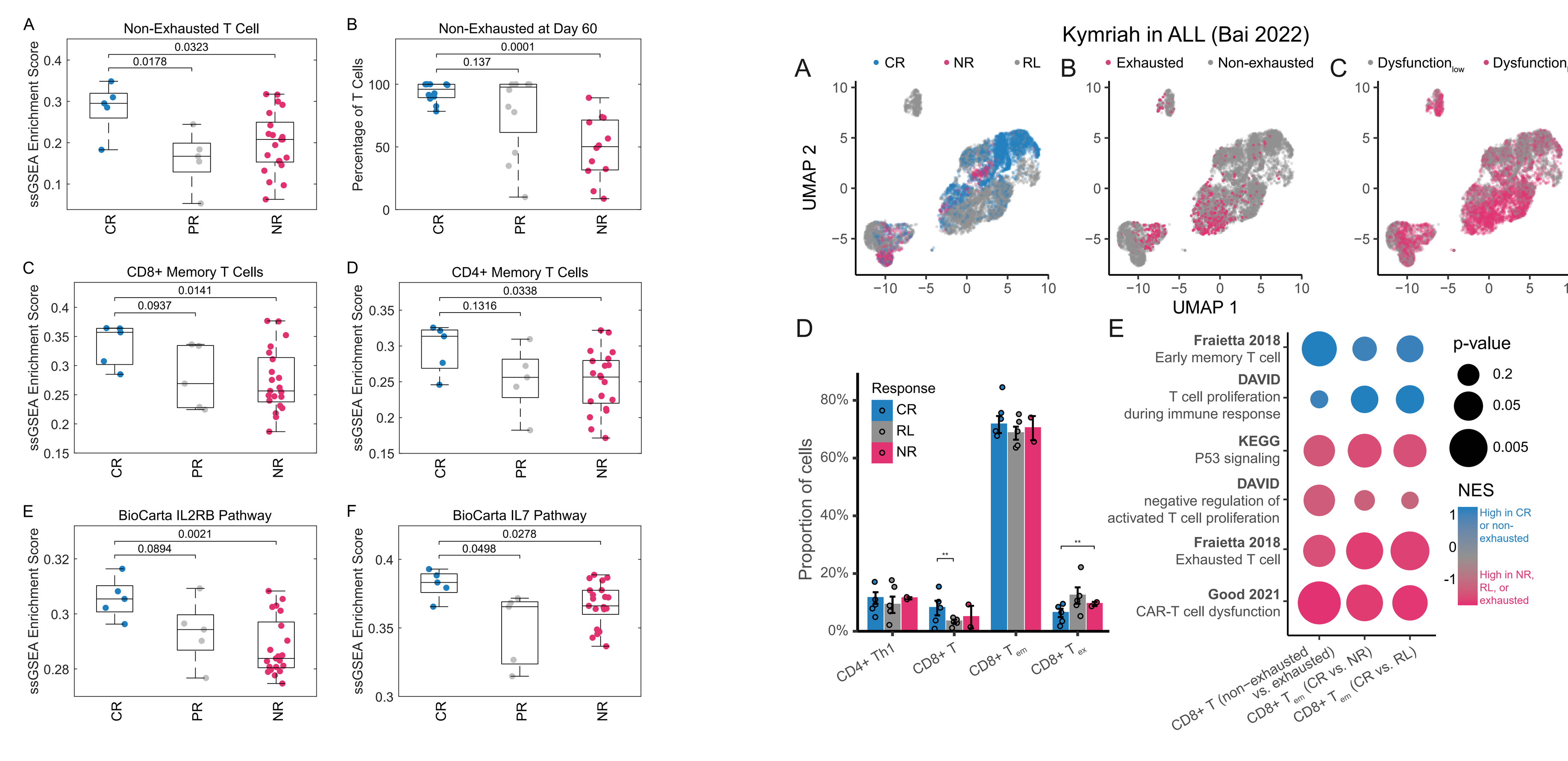
RESULTS

Molecular and cellular features differentiating CR, PR, and NR populations



Single-sample Gene Set Enrichment Analysis (ssGSEA) estimates the activity of signaling pathways and enrichment of cell populations in pre-infusion CAR-T product transcriptomes[1] A,C-F. The CR population is enriched in Non-Exhausted T Cells **A**, matching model simulations **B**, and in T memory cells, confirming model predictions. p-values calculated with a two-sample t-test.

Single-cell RNA sequencing of twelve pre-infusion CAR-T products[4] separated by response. **A** Distinct complete response (CR) non-response (NR), and early relapse (RL) clusters form in UMAP space. Annotation of B exhausted cells using ProjecTILs[6] and C CAR-T cell dysfunction signature.[7] D Cell type frequencies from ProjecTILs show increased CD8+ T cell and decreased CD8+ T exhausted cell proportion in CR (**p<0.05: Wilcoxon rank-sum test). **E** Per-cell type GSEA for selected pathways reveals differential gene signatures, confirming that cell-intrinsic differences exist in pre-infusion product transcriptomes despite similar immunophenotypes across response groups.

Cell-intrinsic attributes predictive of CAR-T response can be inferred from pre-infusion product transcriptomes

Are differences in pre-infusion product transcriptomes predictive of response? A logistic regression-based classifier for the probability of complete response $\mathrm{P}(CR)$ was developed from the ssGSEA scores of the top 28 differentially enriched pathways (CR vs NR) with feature selection via a genetic algorithm:

$$\log\left(\frac{(\mathrm{P}(CR))}{(1-\mathrm{P}(CR))}\right) = \beta_0 + \beta_1 ssGSEA_1 + \cdots + \beta_N ssGSEA_N$$

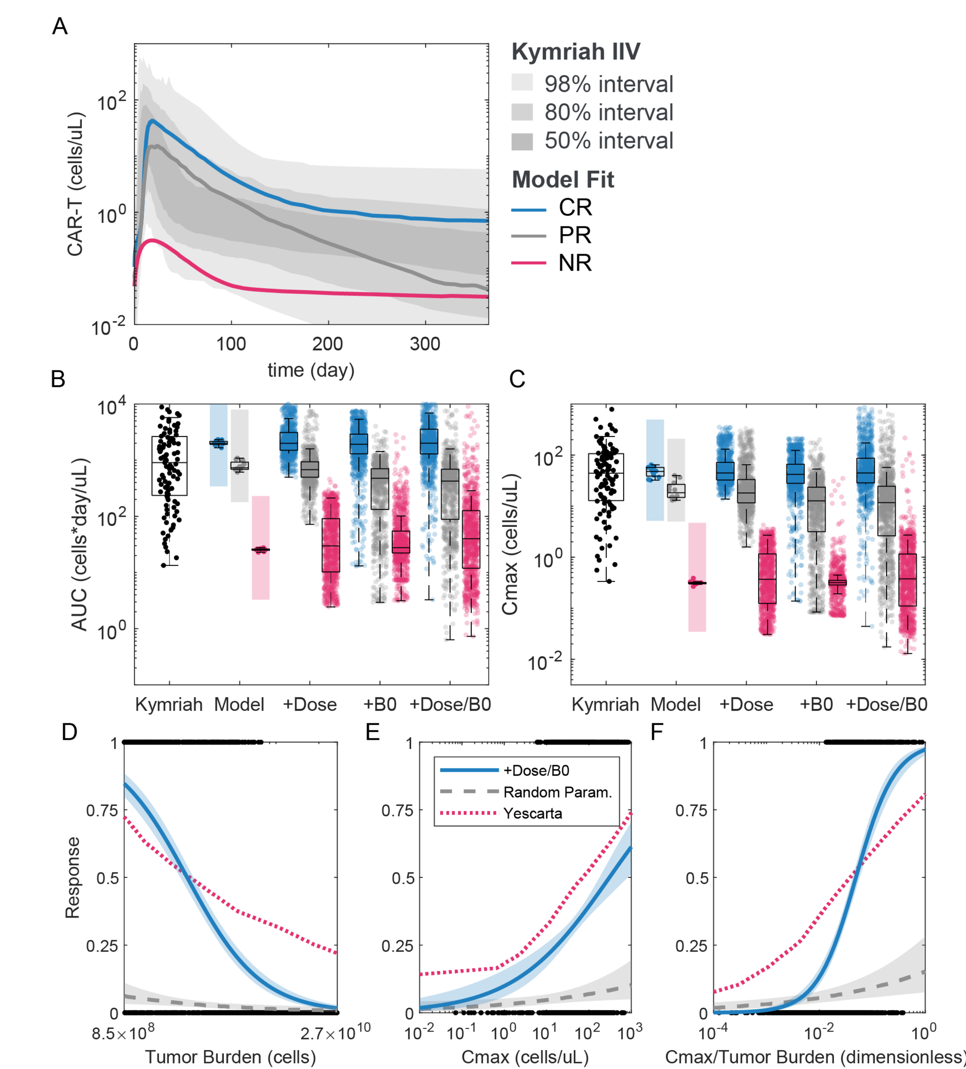
Response can be more accurately predicted with pre-infusion product transcriptomes than immunophenotyping across indications. Distribution of transcriptome-based classifier accuracy from 2500 train-test splits compared to immunophenotype and null (predictions based on the proportion of CR) test models for **A** Kymriah in CLL, B Kymriah in ALL, **C** Kymriah in LBCL, and **D** Yescarta in LBCL. ***: $p \leq 10^{-8}$, Wilcoxon rank-sum test. **E** CART response scorecard, representing the 28 gene signatures fed into the transcriptome classifier, ordered by differential GSEA in Fraietta 2018. Bubble size indicates frequency of inclusion in the 2500 models after feature selection, color indicates differential enrichment between response groups, based on pseudo-bulked GSEA.

RESULTS CONT'D

Explaining inter-patient variability in Kymriah pharmacokinetics

Clinical variability in dose, tumor burden, and CR/PR/NR archetype account for population variance in Kymriah exposure and predict covariates of response to Yescarta.[8]

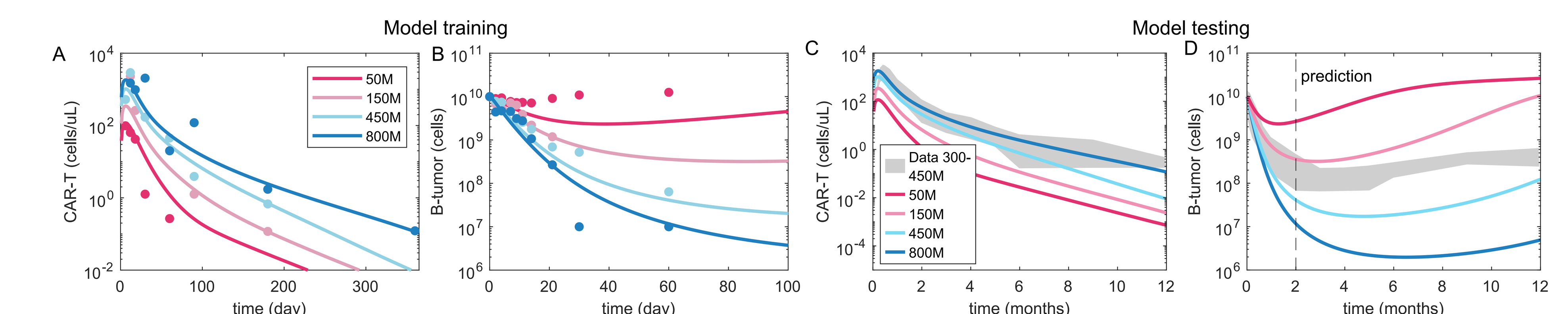
- **A** Model fits to population mean do not account for inter-individual variability (IIV).
- **B-C** Virtual population simulations with random dose ("+Dose") and/or initial tumor burden ("+B0") replicate IIV in AUC and Cmax.
- **D-F** Virtual CR population simulations replicate reported clinical covariates of response to Yescarta in LBCL.



CAR-T dose and initial tumor burden drive clinical variance. Grid simulations reveal a nonlinear relationship between CAR-T dose and initial tumor burden to response (**A** AUC) and exposure (**B** Cmax).

Model extension to Abecma dose response

Application of the modelling framework to a phase I/II dose escalation study of Abecma (a BCMA-targeted CAR-T for multiple myeloma).[9] **A-B** Model training on phase I dose response data. C-D Model testing with predictive simulations matches phase II data beyond initial fitting window.



CONCLUSIONS

- Proliferation of memory cells and cytotoxic potential are cell-intrinsic drivers of response
- Mathematical predictions confirmed via bulk and single-cell RNAseq data analysis
- Pre-infusion transcriptomes are more predictive of response than immunophenotyping
- Variability in CAR-T dose and initial tumor burden fully accounts for inter-patient variability in exposure to Kymriah
- Model simulations could be used to optimize tumor reduction while minimizing Cmax-associated toxicity